



Patent Application of

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Title: Process for the Fractionation of Polymers

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| | 5,567,859 | 10/1996 | Emanuele et al. |
| | 5,605,687 | 5/1992 | Lee |
| 25 | 5,691,387 | 11/1997 | Emanuele et al. |
| | 5,696,298 | 12/1997 | Emanuele et al. |
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This invention claims the priority of provisional application no. 60/225,917 filed on August 17, 2001.

10 **BACKGROUND—Field of Invention**

This invention relates to a process for the fractionation of polymers that are aqueous soluble, and do not contain functional groups capable of carrying a charge at neutral pH.

15

BACKGROUND—Description of Prior Art

Many methods have been described for separating polymers of similar
20 composition and structure. See for example U. S. patents 5,028,336, 5,116,508, 5,523,492, 5,567,859, 5,696,298, 5,800,711 and European Patent Application WO 92/16484, incorporated herein by reference. Also, various procedures have been described to fractionate proteins and peptides, but most include precipitation using ammonium sulfate (Englard and Seifter, 1990). This method
25 relies on the fact that proteins, in an aqueous solution, maintain a tertiary structure based on their amino acid composition and various bonds within the molecule. The tertiary structure generally allows the hydrophobic substituents to be sequestered inside the molecule and the hydrophylic components to be on the surface, and in contact with the aqueous environment. Changes in the ionic
30 strength of the aqueous solution cause unfolding of the molecule, and with exposure of the hydrophobic substituents to the aqueous environment, the solubility of the protein decreases and it precipitates. By carefully adjusting pH, ionic strength, and sometimes temperature, it is frequently possible to separate proteins with similar amino acid sequences (Englard and Seifter, 1990, King,
35 1972, Tarli and Li, 1974). Clearly, this method is useful only for proteins and

5 other polymers composed of substituents that vary considerably in polarity, and therefore aqueous solubility. This is not the case for many synthetic polymers.

A method to separate water soluble organic electrolytes in an aqueous medium from other water soluble hydrocarbons has been disclosed (Bartels and Reale,
10 5,028,336). The pH of the aqueous medium is adjusted so that most of the organic electrolytes are charged. The aqueous medium is then passed through a filtration membrane which carries the same charge. The organic electrolytes are repelled by the charge on the membrane, and therefore do not cross. Water and uncharged organic molecules pass through the membrane and are thus
15 separated from the organic electrolytes. This method is limited to organic molecules such as carboxylic acids which contain functional groups capable of carrying a charge at some pH.

Methods relying on supercritical fluid extraction have been developed to
20 separate high molecular weight compounds, including polymers, from complex mixtures in aqueous solution (See for example Kumar and Hedrick, 5,116,508). This method requires a mobile phase of highly compressed gas, such as CO₂, at or above its critical temperature and pressure, to be pumped through the aqueous solution. The composition of the mobile phase can be modified to
25 enhance extraction of the desired analyte. Such modifications include using a mixture of gases as the mobile phase, or adding a modifying chemical to the supercritical fluid. Such methods can be conducted on a commercial scale, and used to separate uncharged polymers including polyols. Nevertheless, to be effective, the compressed gases often must be maintained at high temperatures
30 and pressures, which requires complex, well controlled equipment. This frequently makes supercritical fluid extraction an expensive process and limits its commercial applications.

Synthetic polyols such as poly(ethylene glycol) and polyoxyalkylene block
35 copolymers have been used in various medical and pharmaceutical applications

- 5 including treatment of sickle cell disease, reduction of blood viscosity, treatment of tissue ischemia, treatment of tissue following electrical injury, and drug delivery (Emanuele 5,691,387, Lee, R.C., 5,605,687, Reeve, L.E., 1997). These linear polymers are generally synthesized by repeated sequential reactions that add monomeric subunits to each end of the polymeric chain.
- 10 Since subunits may add to either or both ends of individual chains at variable rates, the end product is a mixture of molecules varying in molecular weight.

The poly(ethylene glycol)s are composed entirely of ethylene oxide residues linked by ether linkages and vary considerably in molecular weight. These

15 synthetic polymers have been used extensively in drug delivery to solubilize pharmaceutically active compounds. Recently, they have been used to derivatize proteins, peptides and small molecules to prolong half-life and enhance delivery within the body (). They have also been derivatized and used as cross-linking components in medical devices. For optimal safety and efficacy

20 in medical applications, these recent uses require polymers of uniform molecular weight having minimal contamination with reaction byproducts.

The poloxamers are polyoxyalkylene block copolymers composed of two polyoxyethylene blocks separated by a polyoxypropylene center block. The

25 commercially available product contains a mixture of polyoxyethylene homopolymer, and polyoxyethylene/polyoxypropylene diblock polymers in addition to poloxamer molecules of varying molecular weights. These factors cause the polymer product to have a broad molecular weight range, reflected in a high polydispersity index. The mono- and diblock polymers are generally of a

30 lower molecular weight than the average for the polymer product and contain some unsaturation. When commercially available poloxamers (purchased from BASF Corp.) were analyzed by gel permeation chromatography, a bimodal molecular weight distribution was observed (Reeve, L.E., 1997). The mono- and diblock contaminants, including the unsaturated species, partitioned into the

35 lower molecular weight fraction.

European Patent Application WO 92/16484 discloses the use of gel permeation chromatography to isolate a fraction of poloxamer 188 that exhibits beneficial biological effects, without causing potentially deleterious side effects. The copolymer thus obtained had a polydispersity of 1.07 or less, and was substantially saturated. The potentially harmful side effects were shown to be associated with the low molecular weight, unsaturated portion of the polymer, while the medically beneficial effects resided in the uniform higher molecular weight material. Other similarly improved copolymers were obtained by purifying either the polyoxypropylene center block during synthesis of the copolymer, or the copolymer product itself (Emanuele 5,523,492, Emanuele 5,696,298). Although an effective means of purification, gel permeation chromatography is impractical for the preparation of large quantities of the fractionated polyoxyalkylene block copolymer.

A super critical fluid extraction technique has been used to fractionate a polyoxyalkylene block copolymer as disclosed in U.S. Patent No. 5,567,859. A purified fraction was obtained, which was composed of a fairly uniform polyoxyalkylene block copolymer having a polydispersity of less than 1.17. According to this method, the lower molecular weight fraction was removed in a stream of CO₂ maintained at a pressure of 2200 pounds per square inch (psi) and a temperature of 40°C. As is frequently the case, this super critical fluid extraction method required equipment that can control temperature and accommodate compressed CO₂ at high pressure. Clearly, these requirements add expense to the procedure and limit its commercial value.

U.S. Pat. No. 5,800,711 discloses a process for the fractionation of polyoxyalkylene block copolymers by the batchwise removal of low molecular weight species using a salt extraction and liquid phase separation technique. Poloxamer 407 and poloxamer 188 were fractionated by this method. In each case, a copolymer fraction was obtained which had a higher average molecular

5 weight and a lower polydispersity index as compared to the starting material.
However, the changes in polydispersity index were modest and analysis by gel
permeation chromatography indicated that some low molecular weight material
remained. The viscosity of aqueous solutions of the fractionated polymers was
10 significantly greater than the viscosity of the commercially available polymers at
temperatures between 10°C and 37°C, an important property for some medical
and drug delivery applications. Nevertheless, some of the low molecular weight
contaminants of these polymers are thought to cause deleterious side effects
when used inside the body, making it especially important that they be removed
in the fractionation process. As a consequence, polyoxyalkylene block
15 copolymers fractionated by this process are not appropriate for some medical
uses.

Aqueous two phase systems have been used extensively to concentrate or
isolate polymers, other large molecules, and even particles from complex
20 mixtures (Hatti-Kaul, R., 2000). Such systems generally avoid the use of organic
solvents, and extremes of pH or temperature, and, because of their mild
conditions, have been shown to be especially useful to isolate amino acids,
peptides, proteins, plasma membranes including membrane vesicles, and
viruses. These systems are composed of either hydrophilic polymer pairs or a
25 polymer and a salt that are incompatible in aqueous solution and form two
phases in equilibrium with each other. Separations can be carried out using
either batch procedures or counter-current distribution. Although widely used for
the isolation and purification of biomaterials, aqueous two-phase systems have
been used much less extensively for the isolation or fractionation of synthetic
30 polymers.

SUMMARY

The present invention is a method for the fractionation of water-soluble synthetic polymers by physically separating the lower molecular weight polymer molecules, byproducts, and contaminants from the desired polymer by using an aqueous two-phase system.

Objectives and Advantages

The present invention provides a process for the fractionation of aqueous soluble polymers comprising dissolving a known amount of the polymer in water, adding a soluble extraction salt to the polymer solution, maintaining the solution at a constant optimal temperature for a period of time adequate for two distinct phases to appear, and physically separating the phases. Additionally, the phase containing the polymer fraction of the preferred molecular weight may be diluted to the original volume with water, extraction salt added to achieve the original concentration, and the separation process repeated as needed until a polymer having a narrower molecular weight distribution than the starting material and optimal physical characteristics can be recovered.

It is an object of the present invention to provide a method that separates lower molecular weight polyol polymer molecules from those of very similar chemical composition but higher molecular weight thereby narrowing the molecular weight distribution.

It is another object of the present invention to provide a method to remove reaction byproducts from the polyol polymer.

Another object of the present invention is to provide an improved method which produces a polyol polymer with improved chemical and physical characteristics

- 5 including decreased polydispersity, decreased unsaturation, higher viscosity in aqueous solution, and hence increased gel strength, and a well defined transition temperature for those polyols that form gels in solution.

10 **Brief Description of the Drawings**

FIG. 1 is a chromatogram of poly(ethylene glycol) showing the molecular weight distribution of the polymer before and after fractionation using the method of the present invention. The chromatogram was obtained by gel permeation
15 chromatography using two 7.8 mm x 25 cm columns packed with styrene/divinyl benzene copolymer gel (100 μ m pore size) eluted with a solvent composed of 98:2 tetrahydrofuran/triethylamine and a refractive index detector.

FIG. 2a is a chromatogram of poloxamer 407 showing the molecular weight
20 distribution of the polymer before and after fractionation using the method of the present invention. The same chromatographic system described for Figure 1 was used.

FIG. 2b is a comparison of the viscosities of a 25% solution of commercially
25 available poloxamer 407 and fractionated poloxamer 407 obtained from the method of the present invention. Viscosities were measured over a temperature range of about 0°C to about 45°C.

FIG. 3a is a chromatogram of poloxamer 188 showing the molecular weight
30 distribution of the polymer before and after fractionation using the method of the present invention. The chromatographic system described in Figure 1 was used.

FIG. 3b is a comparison of the viscosities of a 35% aqueous solution of
commercially available poloxamer 188 and fractionated poloxamer 188 obtained
35 from the method of the present invention.

5

FIG. 4a is a chromatogram of poloxamine 1307 showing the molecular weight distribution of the polymer before and after fractionation using the method of the present invention. The chromatographic system described for Figure 1 was used.

10

FIG. 4b is a comparison of the viscosities 25% aqueous solution of commercially available poloxamine 1307 and fractionated poloxamine 1307 obtained from the method of the present invention. The viscosities of each solution were measured over a temperature range of about 0°C to about 50°C.

15

Description of the Preferred Embodiments

Although molecular weight averages are informative when comparing polymers, it is also useful to know the molecular weight distribution of each polymer. The polydispersity index of a polymer is a universally accepted measure of the breadth of the molecular weight distribution and is defined as follows:

20

Number Average Molecular Weight (M_n):

25

$$M_n = \frac{\text{Sum of } (W_i) \text{ for all } i \text{ Values}}{\text{Sum of } (W_i/M_i)}$$

30

Weight Average Molecular Weight (M_w):

$$M_w = \frac{\text{Sum of } [(W_i)(M_i)] \text{ for all } i \text{ Values}}{\text{Sum of } (W_i) \text{ for all } i \text{ Values}}$$

35

Polydispersity Index (D):

5

$$(D) = \frac{M_w}{M_n}$$

$$M_i$$

10

where W_i is the mass of the i -th polymer species and M_i is the molecular weight of the i -th species. A low polydispersity value, D , indicates a narrow molecular weight distribution. In a monodisperse population where all molecules are identical, M_w would be equal to M_n and the polydispersity index would be equal to

15 1.0. Typical polymer preparations have polydispersity index values between 1.5 and 5, but some may be much higher.

20

A variety of procedures is available for determining molecular weight including gel permeation chromatography or other chromatographic techniques, viscosity related measurements, light scattering, osmotic pressure, ultra centrifugation and chemical methods involving end group analysis. For most polymers molecular weight distribution parameters including M_w , M_n , and D , are conveniently measured using gel permeation chromatography.

25

The viscosity of a fluid is a measure of friction that results when one layer of the fluid to moves in relation to another layer in response to a shearing force. The terms shear stress (τ) and rate of shear ($\dot{\gamma}$) are used to indicate the applied force and the response of the fluid (Rodriguez, 1989). Shear viscosity is defined as:

30

$$\tau = \text{shear stress} = f/A$$

$$\dot{\gamma} = \text{rate of shear} = u/y$$

$$\eta = \text{shear viscosity} = \tau/\dot{\gamma}$$

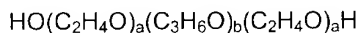
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5 where f/A is the force per unit of area required to maintain a constant velocity gradient, u/y . Viscosity is expressed as Pascal(seconds), or centipoise (cps), where 1000 cps equals 1 Pascal(second).

10 The process of the present invention separates lower molecular weight polymer molecules and byproducts from high molecular weight polyol polymer molecules. The process consists of forming an aqueous two-phase system composed of the polymer and an appropriate salt in water. In such a system, a soluble salt can be added to a single phase polymer-water system to induce phase separation to yield a high salt, low polymer bottom phase, and a low salt, high polymer upper
15 phase. Under carefully selected conditions lower molecular weight polymers partition preferentially into the high salt, low polymer phase. Polymers that can be fractionated using this process include polyethers, glycols such as poly(ethylene glycol) and poly(ethylene oxide)s, polyoxyalkylene block copolymers such as poloxamers, poloxamines, and polyoxypropylene/
20 polyoxybutylene copolymers, and other polyols such as polyvinyl alcohol. The average molecular weight of these polymers may range from about 800 to greater than 100,000 daltons.

The poloxamers are a series of block copolymers having the general structure:

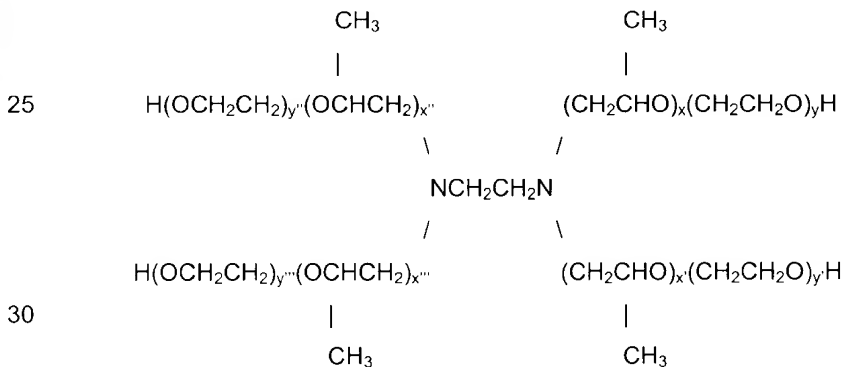
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The average molecular weights of the poloxamers range from about 1,000 to greater than 16,000 daltons. Because the poloxamers are products of a
30 sequential series of reactions, the molecular weights of the individual poloxamer molecules form a statistical distribution about the average molecular weight. In addition, commercially available poloxamers contain substantial amounts of poly(oxyethylene) homopolymer and poly(oxyethylene)/poly(oxypropylene) diblock polymers. The relative amounts of these byproducts increase as the
35 molecular weights of the component blocks of the poloxamer increase.

5 Depending upon the manufacturer, these byproducts may constitute from about
 15 to about 50% of the total mass of the polymer. The process of the present
 invention exploits the differences in size and polarity, and therefore solubility,
 among the poloxamer molecules, the poly(oxyethylene) homopolymer and the
 poly(oxyethylene)/poly(oxypropylene) diblock byproducts. The polar fraction of
 10 the poloxamer, which generally includes the lower molecular weight fraction and
 the byproducts, is removed allowing the higher molecular weight fraction of
 poloxamer to be recovered. The larger molecular weight poloxamer recovered
 by this method has physical characteristics substantially different from the
 starting material or commercially available poloxamer including a higher average
 15 molecular weight, lower polydispersity and a higher viscosity in aqueous solution.

The poloxamines are tetra-functional block copolymers synthesized by the
 sequential addition of first, propylene oxide, and then ethylene oxide to the
 tertiary nitrogens of ethylenediamine. The poloxamines have the following
 20 general structure:



Like the poloxamers, the poloxamines are composed of molecules that vary
 35 considerably in molecular weight. When subjected to gel permeation

5 chromatography, commercially available poloxamine (Tetronic® 1307 purchased from BASF Corp., Mount Olive, NJ) eluted as three separate peaks (Figure 4a). The process of the present invention removed much of the lower molecular weight material, producing a polymer with a slightly higher average molecular weight and more uniform size compared to the starting material. In addition, the
10 viscosities of aqueous solutions of the fractionated polymer were considerably higher than those of the commercially available material.

Because they are composed of hydrophobic poly(oxypropylene) blocks and hydrophilic poly(oxyethylene) blocks, both poloxamers and poloxamines form
15 micelles in aqueous solutions. If the concentration of the polymer is sufficient, the micelles aggregate in a characteristic, temperature dependent fashion, and cause the solution to become a hydrogel. Such hydrogels have been used in various medical applications including site specific drug delivery and manipulation of tissue during and after surgery (Reeve, 1997). Of particular
20 interest are poloxamer 407 and poloxamine 1307 because, for these polymers, the transition from liquid to gel takes place below 37°C. Therefore, in medical applications, a formulation containing these polymers can be applied to the human body as a liquid at or below room temperature that will coat and adhere to tissues, but will rapidly form a gel as it equilibrates to body temperature, and
25 remain where it is placed. However, for both commercially available poloxamer 407 and poloxamine 1307, gelation occurs over a fairly broad temperature range of ten to twenty degrees Celsius. For fractionated polymers recovered from the process of the present invention, in contrast, the transition from liquid to gel occurred in a much narrower, well defined temperature range of approximately
30 two to five degrees Celsius. In addition, the viscosities of gels of various concentrations of either fractionated polymer were higher above 30°C. These two characteristics of the fractionated polymers, rapid transition from liquid to gel over a narrow temperature range, and higher viscosity at body temperature, provide an improved gel for medical applications. Lower concentrations of the
35 fractionated polymer can be used to provide a reliable formulation that will rapidly

5 become a gel at a well defined temperature, but with reduced exposure to the polymer for the patient.

10 The commercially available poly(ethylene glycol)s and poly(ethylene oxide)s are polymers composed of ethylene oxide monomers held together by ether linkages. The poly(ethylene glycols) vary from several hundred to approximately 20,000 average molecular weight. The molecular weights of the poly(ethylene oxide)s range from above 20,000 to several million. Like the polyoxyalkylenes, these polymers are synthesized by the repeated addition of monomers to the ends of the growing polymer chain, and therefore, are composed of molecules
15 varying in molecular weight, and include some relatively low molecular weight material (Figure 1). The process of the present invention was found to remove the lower molecular weight material yielding a more uniform product. The poly(ethylene glycol)s have been used in aqueous two phase systems as the "incompatible" polymer to concentrate and isolate biopolymers. However, the
20 poly(ethylene glycol)s, themselves have not been fractionated or purified by such methods.

The process of the present invention comprises the following steps:

- 25 1. A known amount of the polyol polymer to be fractionated is dissolved in water at an appropriate concentration.
2. The mixture is equilibrated to about 0 to 5°C, then a soluble extraction salt is added slowly with vigorous mixing until the solution becomes opaque. Soluble salts may include salts of sulfate, phosphate and citrate. The
30 preferred salts are sulfates, such as ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$. Dextran and starch may also be used.
3. The solution is allowed to equilibrate at between 0 and 5°C until two distinct phases, upper and lower, appear (usually between about 2 and about 8 hours). Centrifugation may be used to expedite phase separation.

- 5 4. The lower layer is removed. The upper layer is diluted to its original volume
by the addition of deionized water.
5. Steps 2,3, and 4 are repeated from about 2 to about 5 times depending upon
the polyol polymer used as the starting material, the contaminating
byproducts and the degree of fractionation required.
- 10 6. After the final extraction, the upper layer containing the fractionate of the
polyol polymer may be isolated and/or concentrated by extraction into
dichloromethane, chloroform or any other suitable organic solvent or solvent
mixture, or by dialysis. If organic extraction is used, the extract may be dried
using a suitable agent such as anhydrous sodium sulfate.
- 15 7. Residual solvent(s) can be removed by vacuum or lyophilization.
8. The higher molecular weight fraction of the polyol polymer is the dried residue
obtained after removal of the solvent.

20 In the case of the poloxamers and the poloxamines, the fractionated polymer has
a reduced polydispersity index, reduced unsaturation and increased viscosity in
aqueous solution compared to the starting material which allows for better
functionality for various medical and pharmaceutical applications. In the case of
poly(ethylene glycol), the fractionated polymer has a higher average molecular
weight and a narrower molecular weight distribution than the starting material as
25 indicated by a lower polydispersity index.

The following examples illustrate the fractionation process.

30

EXAMPLE 1

Poly(ethylene glycol) (lot number 15628B0) (mwt 10,000) purchased from Aldrich
Chemical Corp., Milwaukee, WI, was weighed and 0.45 grams were dissolved in
35 15 grams of deionized water. The solution was maintained at 1.5°C and 2.51 g

5 of $(\text{NH}_4)_2\text{SO}_4$ were added slowly until the solution turned opaque. Solution was maintained at 2°C , and after two phases formed, the lower phase was removed. Deionized water (13.2 g) was added and the solution was equilibrated at approximately 1°C . Next, $(\text{NH}_4)_2\text{SO}_4$ (2.3 g) was added with stirring until the solution turned opaque. The solution was maintained at approximately 2°C until
10 two phases separated. The lower phase was removed, 12.9 g of deionized water was added, the solution was chilled to 1.3°C , and 2.4 g $(\text{NH}_4)_2\text{SO}_4$ were added. The solution was maintained at approximately 2°C until two phases separated. The upper phase was isolated, washed into a separatory funnel with 30 ml of deionized water and extracted three times with 10 ml dichloromethane.
15 The dichloromethane extracts were combined, and the solvent was removed under vacuum at 20°C . The fractionated polyethylene glycol (0.11 g) was recovered, and found to have an average molecular weight of 11,168 compared to 10,588 for the commercially available poly(ethylene glycol) starting material and a polydispersity index of 1.028 compared to 1.059 for the starting material.

20

EXAMPLE 2

Poloxamer 407 (lot number WPHT-543B), purchased from BASF Corporation, Mount Olive, N.J., was weighed and 486.0 g were dissolved in 15,733 g of
25 deionized water. The solution was maintained at 0.1°C and 2335.1 g of $(\text{NH}_4)_2\text{SO}_4$ were added. The solution was equilibrated at 2°C and after two distinct phases formed, the lower phase was discarded, and the upper phase (2060 g) was collected and weighed. Deionized water (14159 g) was added and the solution was equilibrated to 2°C . Next, 2171.6 g of $(\text{NH}_4)_2\text{SO}_4$ were added
30 with stirring. After the salt was dissolved, the solution was maintained at approximately 2°C until two phases formed. The upper phase (3340 g) was isolated and diluted with 12879 g of deionized water. The solution was chilled to about 2.2°C and 2062 g of $(\text{NH}_4)_2\text{SO}_4$ were added. The phases were allowed to separate as above. The upper phase was isolated and extracted with 4 liters

5 of dichloromethane. Two phases were allowed to form overnight. The organic (lower) phase was isolated and approximately 2 kg of sodium sulfate (Na_2SO_4) were added to it to remove the remaining water. The dichloromethane phase was filtered through a PTFE filter ($0.45\mu\text{m}$ pore size) to remove the undissolved salts. The dichloromethane was removed under vacuum at approximately 30°C .
10 Final traces of dichloromethane were removed by drying in an oven overnight at about 30°C . A total of 297.6 g of fractionated poloxamer 407 (lot number 00115001) were recovered. The chemical and physical characteristics of the fractionated poloxamer 407 are compared to those of the starting material in Table 1.

TABLE 1

| Sample | M_w | M_n | M_w/M_n | Unsaturation mEq/g | Weight % oxyethylene | Viscosity, centipoise* |
|-------------------------------------------------|--------|--------|-----------|-----------------------|-------------------------|---------------------------|
| Poloxamer 407 | 11,996 | 9,979 | 1.20 | 0.048 | 73.2 | 275,000 |
| Poloxamer 407, lot 00115001, fractionated | 13,551 | 12,775 | 1.06 | 0.005 | 69.3 | >820,000 |

*Viscosity of a 25% solution measured at 30°C using a cone and plate viscometer.

EXAMPLE 3

25 Poloxamer 188 (BASF Corp. Lot # WPMO-568B) was weighed and 4.5 g were dissolved in 145.5 g of deionized water. The solution was cooled to 2°C and 26.0 g $(\text{NH}_4)_2\text{SO}_4$ were added and dissolved with stirring. The solution was maintained at approximately 2°C until the phases separated. After two phases formed, the lower phase was discarded, and the upper phase was diluted with
30 125.4 g with deionized water. The solution was cooled to 0.3°C and 20.7g of

5 $(\text{NH}_4)_2\text{SO}_4$ were added slowly with stirring until the solution turned opaque. The solution was maintained at 3°C until two phases formed. After the phases separated, the upper phase was isolated and diluted with 125.4 g with deionized water, chilled to -0.4°C and 21.9 g of $(\text{NH}_4)_2\text{SO}_4$ were added to the solution and dissolved with stirring. The solution turned opaque, and was then maintained at
10 3°C until two clear phases formed. The lower phase was removed, and the upper phase was diluted with 25 ml deionized water. The diluted solution was then extracted three times with 15 ml portions of dichloromethane. The dichloromethane extracts were combined and washed two times with 25 ml portions of deionized water. The water extracts were discarded. The
15 dichloromethane extract was first dried by filtering through anhydrous Na_2SO_4 , and then the solvent was removed under vacuum at 30°C. The remaining solid material was the fractionated poloxamer 188. The fractionated poloxamer 188 was weighed (1.35 g), assigned the lot number 01199001, and its physical properties were evaluated.

20 Both the very high and very low molecular weight material was removed from the fractionated poloxamer 188 (Figure 3a) and the polydispersity was reduced from 1.062 for the unfractionated poloxamer 188 to 1.041. The average molecular weight increased from 7,802 to 8,212. The viscosity of a 35% w/w
25 aqueous solution of fractionated poloxamer 188 began to increase above 35°C, and formed a gel at (and above) 40°C. In contrast, a 35% solution of commercially available poloxamer 188 exhibited an increase in viscosity only above 40°C, and formed a weak gel at approximately 45°C (Figure 3b).

30 EXAMPLE 4

Poloxamine 1307 (BASF Corp, Mount Olive, NJ, Tetronic 1307^(®) Lot No. WPET-587B) was weighed and 0.45 g were dissolved in 15 g deionized water with stirring. The solution was chilled to 1.5°C, and 2.28 g $(\text{NH}_4)_2\text{SO}_4$ were slowly

5 added. The solution was maintained at 2°C until two phases formed. The lower phase was removed, 12.8 g of deionized water were added, and the solution was cooled to 0.6°C. Next, 2.0 g (NH₄)₂SO₄ were added slowly with stirring. The solution was then maintained at 2°C without stirring until two phases formed. The lower phase was removed, 12.7 g of deionized water were added, and the
10 solution was cooled to 1.4°C. (NH₄)₂SO₄ (2.1 g) was added slowly, with stirring until the solution turned opaque. The solution was maintained at 2°C until two phases formed.

The upper phase was isolated and transferred to a separatory funnel with the
15 addition of 30 ml of deionized water. The upper phase was then extracted three times with 10 ml dichloromethane. The dichloromethane extracts were combined, and the solvent was removed under vacuum at 20°C. The resulting solid material (0.19 g) was the fractionated poloxamine.

20 The average molecular weight of the fractionated polymer was 16,217 and the polydispersity was 1.064, compared to an average molecular weight of 14,409 and a polydispersity index of 1.316 for the commercial poloxamine, Tetronic® 1307. A 25% solution of fractionated poloxamine 1307 changed from a liquid to a very stiff gel (viscosity greater than 800 kcps) between 20 and 24°C. In
25 contrast the viscosity of a 25% solution of commercially available poloxamine 1307 began to increase only above 25°C, and formed a non-flowable gel at (and above) 30°C. The maximum viscosity was 494 kcps, and occurred at 40°C (Figure 4b).

30 These examples should not be construed as limiting the scope of the invention, but as merely providing illustrations of some of the presently preferred embodiments to this invention.